concentrations of human recombinant Il-1β (5 ng/ml) and TNFα (20 or 40 ng/ml) and simultaneously with etanercept at 100 μg/ml for 24 hours; gene expression of iNOS Il-6, and COX-2 and was assessed by the RT/PCR method using 18S rRNA as the housekeeping gene.
Results: Etanercept determined a complete suppression of TNFα-induced iNOS, Il-6, and COX-2 gene expression. This anti-TNFα drug also downregulated, with a dose-dependent effect, iNOS and COX-2 gene expression (−20% and −30% respectively, $p < 0.05$) after stimulation with Il-1β. Etanercept seemed to have no effects on Il-1β-induced Il-6 gene expression.
Conclusions: To our knowledge, this is the first report of the effects of etanercept on iNOS, Il-6 and COX-2 gene expression, after inflammatory stimulation, on a human chondrocyte population. In summary, these data suggest that the beneficial effects of the treatment with etanecerpt in EOA could be further explained by the action of this biological drug at cartilage level.

221 COLLAGEN TYPE IX AND CARTILAGE OLIGOMERIC MATRIX PROTEIN INFLUENCE CARTILAGE MATRIX ASSEMBLY

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Purpose: Collagen type IX (COL9) and Cartilage Oligomeric Matrix Protein (COMP) are proteins present in the cartilage matrix. Even though there is clear evidence that these cartilage matrix proteins interact with each other and collagen type II, their exact function in matrix organisation is not yet completely understood. In early osteoarthritic cartilage, collagen type II expression is increased but repair is ineffective. Other matrix components such as COL9 and COMP are possibly required for the formation of the collagen fibrils and the structure and integrity of the network. To get more insight into these requirements and investigate them as possible targets for matrix regeneration, we investigated the role of COMP and collagen type IX in cartilage matrix production.
Methods: Chondrocytes isolated from mice deficient in COL9 and COMP (double knock-out, DKO), COL9 alone (Col9−/−) and wild type (WT) mice were cultured for five weeks in alginate beads. Collagen cross-linking (specifically the HP cross-links), glycosaminoglycan (GAG) and collagen deposition and distribution over cell-associated, further-removed matrix, and culture medium were determined.
Results: In both knock-out conditions, less GAG was deposited than in the WT condition: 10.2±0.3 μg GAG/bead in the WT condition, 5.9±0.1 μg/bead in Col9−/− and 6.4±2.9 μg/bead in the DKO condition. Collagen deposition was only less in Col9−/−: 6.3±0.9 μg collagen/bead in the WT condition versus 3.9±0.4 μg/bead in Col9−/−. The absence of COL9 and COMP differentially influenced collagen and GAG retention and distribution within the alginate bead. In both knock-out conditions, less GAG and collagen was present in the cell associated matrix (CM) than in the control condition. GAG and collagen distributed differentially over the further removed matrix (FRM) and the culture medium (Figure 1). In addition, the deposited collagen was less cross-linked in both knock-out conditions: 0.52±0.02 HP/collagen in the control condition, 0.39±0.01 HP/collagen in Col9−/− and 0.43±0.00 HP/collagen in DKO.

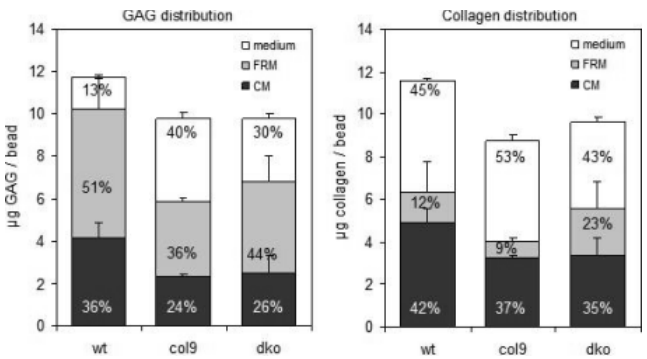

Figure 1: Distribution of extracellular matrix components between the cell associated matrix (CM, black), further removed matrix (FRM, light grey) and the culture medium (white) in cultures of cells deficient for COL9 or COL9 and COMP (dko). The bars represent the absolute quantity with the relative distribution shown as percentages.

Conclusions: COL9 and COL9/COMP deficiency resulted in less cartilage matrix deposition and altered distribution, suggesting that the effects seen are a result of COL9 deficiency. COMP deficiency in addition does not change the effect seen with single COL9 knockout. COL9 seems more important in the formation of a functional cartilage matrix than COMP and might therefore be a target in cartilage regeneration.

222 NITRIC OXIDE (NO) IN SOME CONDITIONS INDUCES AUTOPHAGY IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Nitric Oxide (NO) is a short-lived and multifunctional molecule that mediates various biological processes. Osteoarthritis disease (OA) is characterized by an increase in both chondrocyte death (apoptosis) and NO production; in fact, OA cells produce as much, if not more, NO than any other cell in the body. Different studies have shown up until now that programmed cell death is not necessarily synonymous with apoptosis. It has been described that in the growth plate of cartilage, stress conditions can promote an autophagic response in chondrocytes, through the regulation of genes controlling metabolite utilization. Besides serving a cytoprotective role, it is known that autophagy can function in cell death. Articular cartilage shows per se hostile conditions so it is an avascular tissue and possesses low O_2 tensions.
Objective: To study the induction of autophagic features in normal and OA human chondrocytes by NO.
Methods: Normal and OA human cartilages were obtained from patients with joint replacement (femoral and knee joint) and from autopsy cases (knee joint). After enzimatic digestion, chondrocytes were kept in DMEM with 10% SBF at 37ºC in a humidified atmosphere provided by an incubator until the first subculture was reached. Normal, OA and NO donors (SNP and NOC-12) treated normal cells were analyzed by flow cytometry to quantify the apoptosis by means of propidium iodide and BRDU methods. On the other hand, the expression of two AuTophaGy-related (*ATG*) genes, Beclin-1 and APG-7, was assessed by means of western-blot, immunocytochemistry and flow cytometry, using specific monoclonal antibodies (Abcam, UK). Besides, we analyzed the β-galactosidase (an enzyme linked to lysosome) activity in cells by means of a commercial kit (BioVision, Inc).
Results: Previous results obtained by us showed that normal and OA chondrocytes show morphologic changes more characteristic of autophagocytosis than apoptosis. Direct observation with optic microscopy of chondrocytes treated with NO donors stated the remarkable morphological differences between SNP induced effects (apoptotic typical morphology) and NOC-12 induced effects (apparition of striking cytoplasmatic vacuoles). Results obtained by means of flow cytometry, both using the propidium iodide method as well as the highly specific BRDU method, showed us that apoptotic cell percentage induced by classic NO donor SNP (~45%) is much higher than the percentage observed with NOC-12 (~11%). The study of Beclin-1 expression by means of western-blot showed us that in a significant way OA chondrocytes have a 1.7 ratio in the expression of this protein with respect to the normal cells, findings were corroborated with an immunocytochemistry study; however, APG-7 levels were similar between normal and OA chondrocytes, although we could observe that the latter ones show a crescent tendency in its expression by means of flow cytometry. On the other hand, both NOC-12 and SNP treated chondrocytes show Beclin-1 and APG-7 expression. We observed β-galactosidase activity in all cases.
Conclusions: These results show that, besides apoptosis, programmed cell death-Type II is important to human articular chondrocytes. The presence of autophagic levels in human chondrocytes, suggest to us a response that can permit the terminally differentiated cells to survive the brief rigors of the harsh local microenvironment that exists in human cartilage, all above in OA cartilage.

223 EFFICIENT NON-VIRAL TRANSFECTION OF PRIMARY HUMAN ADULT CHONDROCYTES AND CHONDROCYTE-DERIVED CELL LINES IN A HIGH-THROUGHPUT FORMAT

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Purpose: The in vitro transfer of plasmid DNA or siRNA constructs into cells provides a powerful tool for the analysis of basic cellular and